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Short communication

Drought responses of root biomass provide an indicator of soil microbial drought resistance in grass monocultures

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ABSTRACT

Plant species may exert a strong influence on soil biological properties, but the linkages between plant and soil responses to severe drought remain unclear. We conducted an outdoor mesocosm experiment with five upland grass species and one Mediterranean drought-resistant grass cultivar to investigate the effects of root biomass and rhizosphere conditions on the drought responses of soil microbial biomass in the topsoil. In particular, we assessed whether variation in the drought resistance of microbial biomass could be linked to root biomass, soil inorganic nitrogen (N) or dissolved organic carbon (DOC). Experimental drought decreased microbial biomass but increased soil inorganic N and DOC across plant species. Root biomass responses to drought were less predictable, and varied depending on species. Microbial biomass resistance to drought showed a negative relationship with the drought resistance of root biomass across species, possibly via changes in rhizodeposition. Moreover, the drought resistance of microbial biomass showed a negative relationship with soil nutrient availability under droughted conditions. Our findings highlight the importance of root biomass as a predictor of soil microbial resistance to drought in grass-dominated systems, and suggest that trade-offs between plant and microbial processes could have significant implications for ecosystem function in a changing environment.

1. Introduction

The soil microbiota plays a key role for biogeochemical cycling, with cascading effects on primary production and biodiversity as well as climate-ecosystem feedbacks (Wall, 2012). However, the capacity of soil microorganisms to maintain soil function and ecosystem services faces threats from changing management practices and increasingly-common drought events associated with climate change (Griffiths and Philippot, 2013; Rivest et al., 2015). Unravelling the mechanisms underlying soil microbial responses to environmental stress is critical for the improved prediction of soil function in a changing environment.

Microbial resistance to environmental stress is generally thought to be driven by a variety of abiotic factors including soil organic matter, nutrient availability, pH and soil aggregation which impact microbial community composition and microbial activity (Griffiths and Philippot, 2013). These soil properties may themselves be shaped by the resident plant community *via* species-specific differences in root exudate production, nutrient uptake or litter inputs (Bardgett et al., 1999; Singh et al., 2009). Growing evidence suggests that the study of plant traits could be a powerful approach for exploring the complexity of plant species effects on soil processes (Bardgett et al., 2014; Cantarel et al., 2015; de Vries et al., 2016).

Grassland studies under non-stressful conditions have shown linkages between plant root traits and soil microbial communities (Valé et al., 2005; Orwin et al., 2010; Legay et al., 2014), supporting the idea that trait-mediated changes in the quality and quantity of root exudates and rhizodeposits explain variation in microbial community function (Warembourg et al., 2003). Root-derived carbon (C) sources may also condition microbial drought responses if high soil C availability promotes fast-growing, drought-resilient microbes at the expense of slow-growing, drought-resistant microbes (Orwin and Wardle, 2005; de Vries and Shade, 2013). Comparisons of different plant functional groups (grasses, legumes, forbs) indicate that root biomass affects microbial community structure and function under droughted conditions (Orwin and Wardle, 2005; de Vries et al., 2016). To date, however, the influence of plant root biomass on microbial drought resistance has never explicitly been tested for multiple grassland species within the same plant functional group.

Here we report a mesocosm study on the interactive effects of grass species identity and severe summer drought on microbial biomass C, soil inorganic nitrogen (N) and dissolved organic C. Measurements were carried out at the end of drought manipulation to assess the linkages between microbial biomass resistance to drought, soil nutrient availability and plant root biomass. We predicted that soil responses to a

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severe summer drought would vary depending on plant species, and tested the hypothesis that variation in plant root biomass is closely related to microbial drought resistance.

2. Materials and methods

2.1. Experimental design

The mesocosm experiment was conducted at INRA, Clermont Ferrand (45°47′N, 03°05′E, 350 m a.s.l.), and comprised of two treatments in a fully factorial design: grass species monocultures (five natives, one cultivar) and rainfall treatment (well-watered, severe summer drought). We chose five grass species common in semi-natural, mesic grasslands which are known to vary in terms of biomass production and plant traits in well-watered and drought conditions (Pontes et al., 2010; Zwicke et al. 2015) i.e. *Dactylis glomerata*, *Festuca arundinacea*, *Poa pratensis*, *Poa trivialis* and *Trisetum flavescens*. In addition we used one Mediterranean cultivar of *Dactylis glomerata* characterised by high drought survival (cultivar Medly, RAGT, France). Droughted mesocosms were replicated four times per species whereas well-watered mesocosms were replicated three times per species, resulting in a total of 42 mesocosms. The uneven replicate number across drought treatments was due to logistical constraints (lack of space); we chose to increase the number of replicates in the treatment with strong water stress in case of high plant mortality.

In September 2010, experimental mesocosms (stainless steel free-draining boxes, 50 × 50 × 40 cm) were filled with 100 L of topsoil (20.8% clay, 19.7% silt, 59.5% sand, 4.3% organic matter) extracted from a nearby grassland. Soil was mixed with slow-release fertiliser (3.5 kg m^{-3}, NPK 14-7-14, Multicote 12, Haifa, Israel) to promote plant growth. Mesocosms were insulated with a 50 mm layer of polystyrene (Styrodur®, BASF, France) to minimise soil warming. Seeds of each study species were sown into experimental mesocosms at a density of 2000 m^{-2}, and mesocosms were kept close to field-carrying capacity by regular watering. Plants were left to grow outside until the application of rainfall treatments in July 2011, and maintained in a vegetative state by regular cutting to a height of 5 cm (cuts in April, May and June; last cut on the 15th June). Cutting management was in line with local cutting practices for productive grasslands. Severe drought was simulated from the 1st July until 2nd August 2011 (32 days drought duration) by stopping irrigation and intercepting precipitation with a transparent polycarbonate shelter (12.5 × 10.8 m, 6.2 m high, 90% transmitted PAR, Batiroc, France). The shelter was automatically controlled by a rain sensor, and was only maintained over 'drought' mesocosms during rainy weather conditions to minimize treatment artefacts. The remaining mesocosms were well-watered and maintained close to field-carrying capacity throughout the experimental period (following Zwicke et al., 2015).

2.2. Plant and soil measurements at the end of drought

At the end of the experimental drought, one intact soil core (10 cm diameter, 0–15 cm deep) was taken from the centre of each mesocosm. Soil cores were sieved (2 mm mesh) and root biomass was determined for each core; root samples were washed then oven-dried at 60 °C for 48 h and weighed. Soil mineral N was extracted from a sub-sample of freshly-sieved soil by shaking 5 g of soil with 25 mL 1 M KCl for 1 h on an orbital shaker. The KCl extracts were filtered through Whatman glass microfibre filters and analyzed by colorimetric measurements (Bran & Luebbe Auto Analyser 3, Hamburg, Germany). Microbial biomass C was measured on 5 g subsamples of freshly sieved soil using the chloroform fumigation–incubation method (Brookes et al., 1985). In brief, soluble C was extracted from fumigated and unfumigated samples with 25 mL of 0.5 M K_2SO_4 solution and determined by high temperature catalytic combustion (SkalarFormacs CA14 analyzer, Skalar Analytical B.V., Breda, The Netherlands). Microbial C was calculated as the difference in total C extracted in fumigated and unfumigated soils, with $k_C = 0.35$ as the adjustment factor (Sparling et al., 1990). Non-fumigated extracts were used as an estimate of dissolved organic C (K_2SO_4-extractable DOC) following Bloor and Bardgett (2012). Additional sieved soil subsamples were oven-dried (105 °C, 24 h) to determine soil water content per soil core.

2.3. Statistical analyses

Treatment effects on plant and soil variables were analysed using analysis of variance (ANOVA). When the ANOVA was significant, post hoc analysis was performed. Drought resistance of microbial and root biomass was calculated as: $\frac{D}{C}$ following Griffiths and Philippot (2013), where D is the performance under droughted relative to control (C) conditions at the end of drought. Regression analysis was used to identify linear relationships between drought responses in microbial biomass, root biomass and soil properties across species. We also used PCA to derive a multivariate index of soil nutrient availability under droughted conditions based on inorganic N, DOC and root biomass (i.e. scores for the first principal components axis, Fig. A1). All data were checked to meet assumptions of normality and homogeneity, and statistical analysis was carried out using Statgraphics Plus 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA).

3. Results

Experimental drought had a strong negative effect on soil moisture irrespective of plant species (−69.5 ± 0.80% on average across species, $F_{1,29} = 6218.5$, $P < 0.001$, data not shown), and caused complete senescence of aboveground shoots. In general, drought decreased microbial biomass C but increased soil inorganic N and DOC (Table 1, Fig. 1). The magnitude of drought-induced changes to both microbial biomass C and DOC varied depending on species identity (Table 1), with greatest changes observed for *Trisetum* (Fig. 1). Root biomass responses to drought also varied depending on species (significant species × drought interaction, Table 1). Grass species could be broadly classed into three groups depending on their root biomass response (Fig. 1): decreased biomass when exposed to drought (*Dactylis glomerata*, *Poa pratensis*, *Poa trivialis*), no clear biomass response to drought (*D. glomerata* cv *Medly*, *Festuca arundinacea*) and increased root biomass in droughted mesocosms (*Trisetum flavescens*).

Regression analysis revealed a negative relationship between the drought resistance of microbial biomass C and root biomass across

Table 1
Effects of plant species and drought treatment on soil variables recorded in the 0–15 cm soil layer. Values shown are probabilities associated with the F-ratio (ANOVA); significant effects ($P < 0.05$) are shown in bold type.

Variable	Effect[†]		
	Species	Drought	Species × Drought
K_2SO_4-extractable DOC (mg DOC g^{-1})	**0.03**	< **0.001**	**0.038**
Soil inorganic N (μg N g^{-1})	0.91	< **0.001**	0.72
Microbial biomass C (μg C g^{-1})	**0.002**	< **0.001**	**0.003**
Root biomass (g m^{-2})	**0.012**	0.29	**0.035**

[†] $DF_{5,29}$ for all terms except drought ($DF_{1,29}$); n = 4.

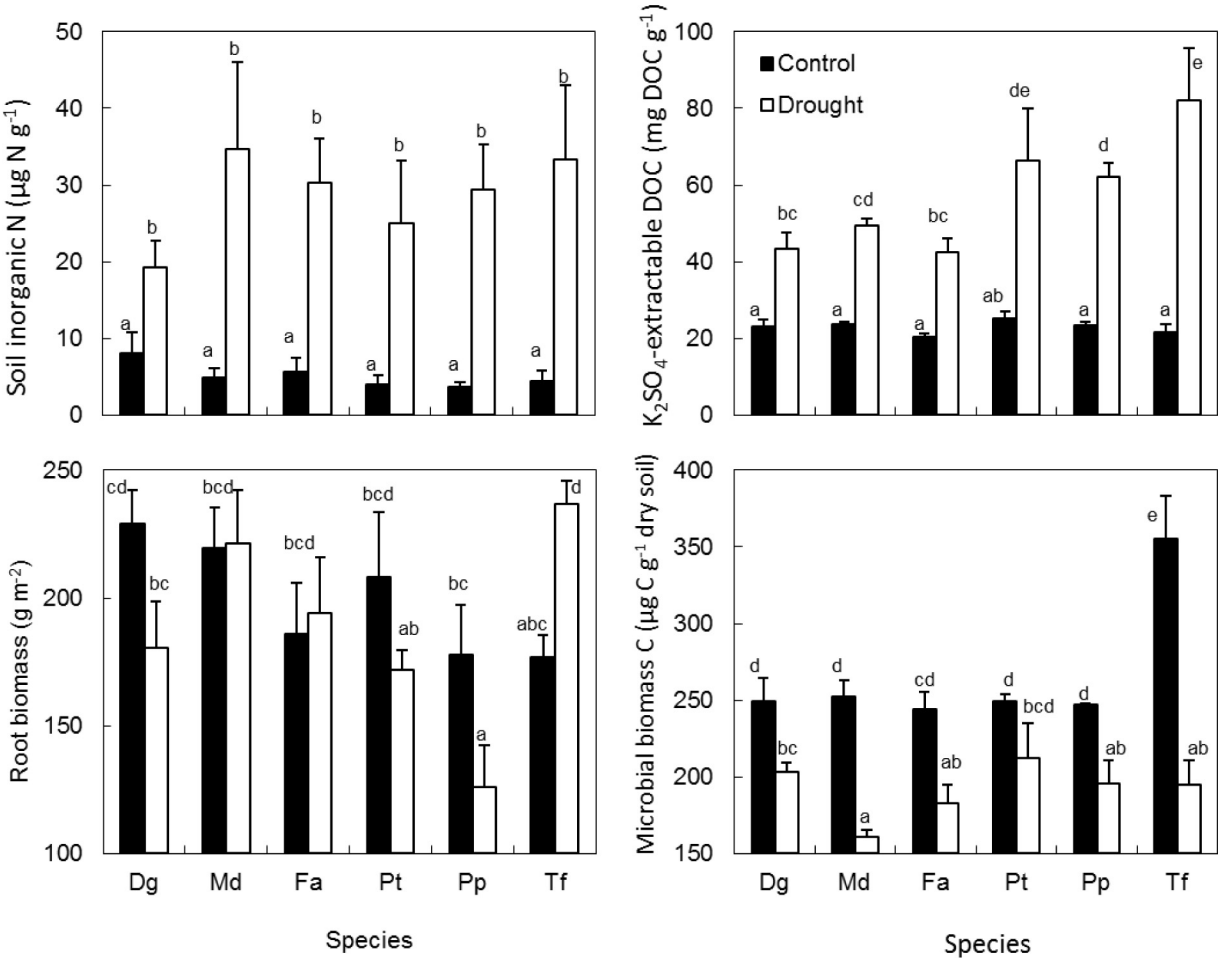

Fig. 1. Effects of plant species and drought treatments on soil variables in the 0–15 cm soil layer at the end of experimental drought. Species codes are given by: *Dactylis glomerata*, Dg; *Dactylis glomerata* cultivar Medly, Md; *Festuca arundinacea*, Fa; *Poa trivialis*, Pt; *Poa pratensis*, Pp; *Trisetum flavescens*, Tf. Means (+1 standard error) are shown; means not sharing the same letter are significantly different at $P < 0.05$ (post hoc tests).

study species ($r^2 = 0.73$, $P = 0.018$; Fig. 2). Microbial biomass resistance also showed a negative relationship with the multivariate soil nutrient index under droughted conditions (i.e. PC1 scores driven by soil inorganic N, $r^2 = 0.80$, $P = 0.011$; Fig. 2). Neither microbial biomass drought resistance nor microbial biomass C showed any significant relationship with single soil properties.

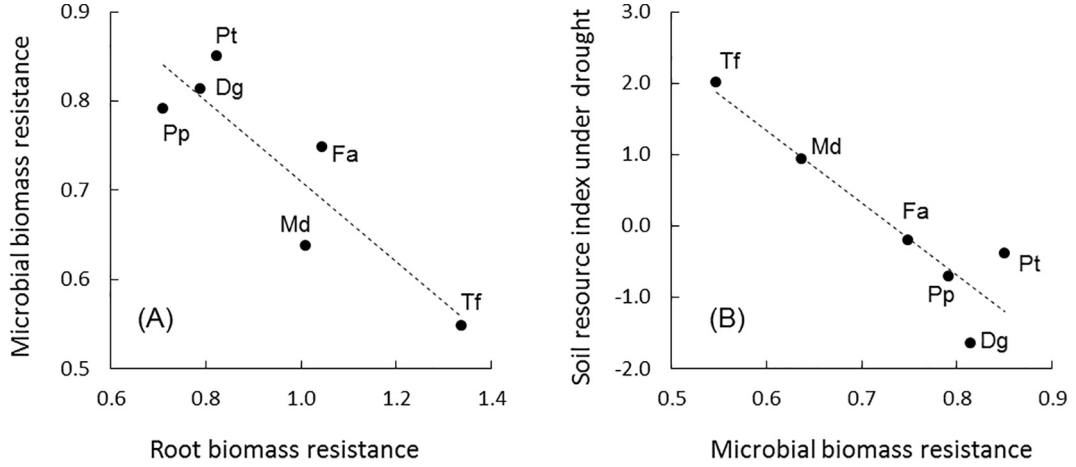

Fig. 2. Relationships between microbial resistance to drought and (A) root biomass drought response, or (B) soil nutrient resource availability in droughted conditions. Values for resistance are response ratios; values for soil nutrient availability are PC1 scores from a PCA biplot based on soil inorganic N content, DOC and root biomass. The dashed lines represent linear regressions ($P < 0.05$). Species codes as in Fig. 1.

4. Discussion

Severe drought events often have strong impacts on soil processes and biogeochemical cycling (Bloor and Bardgett, 2012; Sanaullah et al., 2012; but see Kreyling et al., 2008). In the present study, drought decreased microbial biomass C but increased soil inorganic N and DOC across plant species. Observed drought-induced increases in DOC almost certainly reflect a combination of processes: increased microbial death (consistent with our microbial biomass data), shifts in microbial C use (Fuchslueger et al., 2014) and increases in fine root turnover, or leakage of cell contents from damaged tissue (Henry et al., 2007; Sanaullah et al., 2012; Zwicke et al., 2015). Interestingly DOC showed significant interactions between species and drought, with smaller drought-induced increases in DOC recorded for the tall, deep-rooted species (*Dactylis* and *Festuca*). Drought-induced increases in soil mineral N agree with results reported elsewhere in multi-species experiments, and are thought to be driven by asynchrony in plant and soil process rates during drought i.e. modified source/sink relationships for N (Bloor and Bardgett, 2012).

As expected, the magnitude of drought response in microbial biomass varied across plant species. These data confirm previous findings of significant plant species identity effects on soil properties under drought (Orwin and Wardle, 2005; Rivest et al., 2015; but see de Vries et al., 2016). Data from this experiment also support our main hypothesis since species effects on microbial drought resistance were linked to variation in the drought response of root biomass in the topsoil (0–15 cm soil layer). Microbial drought resistance showed a trade-off with root biomass resistance; microbial biomass resistance was high where root biomass showed a strong negative response to drought, whereas microbial biomass resistance was low where root biomass showed a positive response to drought. Microbial drought resistance also occurred at the expense of soil resource availability under droughted conditions (PC1 scores, correlated with soil inorganic N). Low microbial resistance suggests a microbial community dominated by fast-growing r-strategists (de Vries and Shade, 2013). Transient increases in soil resource availability with low microbial drought resistance could translate to drought-induced shifts in plant/microbial competition for N (Hodge et al., 2000), with implications for nutrient cycling and plant regrowth following rewetting (Roy et al., 2016).

Assuming that drought-induced cessation of root growth decreases rhizodeposition rates, our results are consistent with the idea that low soil C availability (and associated changes in microbial community structure) promotes microbial resistance to drought (Orwin and Wardle, 2005). Our data also confirm that feedbacks and trade-offs between plant- and soil processes could play a significant role for soil function and provision of ecosystem services under drought, as suggested elsewhere (Bloor and Bardgett, 2012; Roy et al., 2016). However, we found that DOC itself was not a good predictor of microbial drought resistance, possibly due to confounding effects of microbial death under drought.

Overall, our results demonstrate that drought responses in root biomass are a useful predictor of soil microbial sensitivity to drought for grassland monocultures and plant species within the same functional group. Our data also suggest a possible trade-off between plant-mediated changes in the rhizospheric conditions and microbial community stability under severe drought. These findings need to be confirmed for natural grasslands where grasses grow in mixtures with other plant functional groups as well as different grass species. Future studies should also examine the influence of root exudation and physiological root traits on the drought resistance and resilience of different microbial functional groups to better understand the underlying mechanisms of microbial community responses.

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Appendix

See Fig. A1.

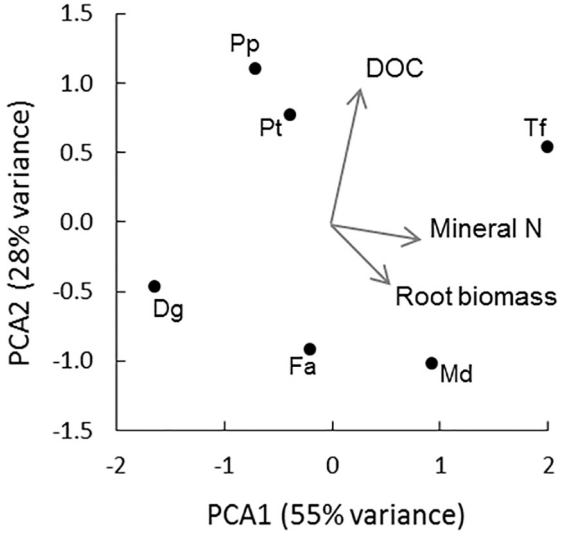

Fig. A1. PCA biplot based on soil inorganic N content, DOC and root biomass measured at the end of experimental drought in droughted mesocosms. Symbols represent mean values for species treatments. Species codes are given by: *Dactylis glomerata*, Dg; *Dactylis glomerata* cultivar Medly, Md; *Festuca arundinacea*, Fa; *Poa trivialis*, Pt; *Poa pratensis*, Pp; *Trisetum flavescens*, Tf.

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